



U.S.S.N. 09/714,802
Filed: November 16, 2000

CLEAN VERSION OF AMENDMENTS PURSUANT TO 37 C.F.R. § 1.121

Clean Version of Amended Claims

Pursuant to 37 C.F.R. § 1.121(c)(1)(ii)

3. (Amended) The method according to Claim 57 further comprising after step (a), removing auxotrophs from the plurality of mutant microorganisms.

57. A method for identifying a mutant microorganism having a reduced adaptation to a particular environment comprising the steps of

(a) providing a plurality of mutant microorganisms wherein each microorganism contains a different marker sequence;

(b) introducing the plurality of mutants of step (a) into the said particular environment and allowing those microorganisms which are able to do so to grow in the said environment;

(c) retrieving microorganisms from the said environment or a selected part thereof; and

(d) selecting an individual mutant having a reduced capacity to proliferate in the particular environment by comparing any marker sequences in the nucleic acid present in the retrieved microorganisms in step (c) to the different marker sequences referred to in step (a).

58. The method of Claim 57 for identifying a gene which allows a microorganism to adapt to a particular environment further comprising the step:

(e) identifying the gene which is mutated in the individual mutant having a reduced capacity to proliferate in the particular environment.

59. The method of Claim 58 for isolating a gene which allows a microorganism to adapt to a particular environment further comprising the step:

(f) isolating from a wild-type microorganism the corresponding wild-type gene.

60. The method of Claim 59 wherein the particular environment is a differentiated multicellular organism.

61. The method of Claim 60 wherein the multicellular organism is a plant.

62. The method of Claim 61 wherein the microorganism is a bacterium pathogenic to plants.

63. The method of Claim 61 wherein the microorganism is a fungus pathogenic to plants.

64. The method of Claim 60 wherein the multicellular organism is a non-human animal.

65. The method of Claim 64 wherein the animal is selected from the group consisting of a mouse, rat, rabbit, dog and monkey.

66. The method of Claim 65 wherein the animal is a mouse.

67. The method of Claim 64 wherein the microorganisms is a fungus pathogenic to animals.

68. The method of Claim 67 wherein the fungus is selected from the group consisting of *Aspergillus* spp., *Cryptococcus neoformans* and *Histoplasma capsulatum*.

69. The method of Claim 64 wherein in step (b) the microorganisms are introduced orally, intravenously, intranasally, or intraperitoneally.

70. The method of Claim 69 wherein in step (c) the microorganisms are retrieved from the spleen.

71. The method of Claim 64 wherein the microorganism is a bacterium pathogenic to animals.

72. The method of Claim 71 wherein the bacterium is selected from the group consisting of *Bordetella pertussis*, *Campylobacter jejuni*, *Clostridium botulinum*, *Escherichia coli*, *Haemophilus decreyi*, *Haemophilus influenzae*, *Helicobacter pylori*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Listeria* spp., *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pseudomonas* spp., *Salmonella* spp., *Shigella* spp., *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Vibrio* spp., and *Yersinia pestis*.

73. The method of Claim 60 wherein in step (c) the microorganisms are retrieved from the said environment at a site remote from the site of introduction in step (b).

76. The method of Claim 57 wherein the microorganism is a bacterium.

77. The method of Claim 57 wherein the microorganism is a fungus.

78. The method of Claim 57 wherein in step (d) the comparison of any marker sequences in the nucleic acid of the mutants retrieved in step (c) to the marker sequences referred to in step (a) uses DNA amplification techniques and oligonucleotide primers.

80. A non-human animal or plant, or an animal or plant cell culture, containing a plurality of mutant microorganisms wherein each mutant contains a different marker sequence.

81. The non-human animal or plant, or an animal or plant cell culture, of Claim 80 wherein the microorganism is a pathogenic microorganism.

82. A non-human animal or an animal cell culture containing a plurality of mutant

microorganisms wherein each mutant contains a different marker sequence and wherein the microorganism is pathogenic to animals.

83. The non-human animal or an animal cell culture of Claim 82 wherein the microorganism is selected from the group consisting of *Bordetella pertussis*, *Campylobacter jejuni*, *Clostridium botulinum*, *Escherichia coli*, *Haemophilus ducreyi*, *Haemophilus influenzae*, *Helicobacter pylori*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Listeria* spp., *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pseudomonas* spp., *Salmonella* spp., *Shigella* spp., *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Vibrio* spp., and *Yersinia pestis*.

84. The non-human animal of Claim 83 which is a mouse or rat or rabbit or dog or monkey.

85. The non-human animal of Claim 82 which is a mouse or rat or rabbit or dog or monkey.

86. A method for identifying a microorganism having a reduced adaptation to a particular environment comprising the steps of

(a) providing a plurality of microorganisms wherein each microorganism contains a different marker sequence;

(b) introducing the plurality of microorganisms of step (a) into the said particular environment and allowing those microorganisms which are able to do so to grow in the said environment;

(c) retrieving microorganisms from the said environment or a selected part thereof; and

(d) selecting an individual microorganism having a reduced capacity to proliferate in the particular environment by comparing any marker sequences in the nucleic acid present in the retrieved microorganisms in step (c) to the different marker sequences referred to in step (a).

ATL1 #562797 v1